Effects of Peroxide, Catalase, and Hematin in the Assay of Liver Tryptophan Pyrrolase*

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Continuous assay of the soluble and inducible tryptophan pyrrolase (L-tryptophan : H₂O₂ oxidoreductase, EC 1.11.1.4) by direct spectrophotometry in the particle-free supernatant fraction of liver (1) would be a desirable alternative to the usual assay in whole liver homogenates (2) for studies of the adaptive variations of the concentration of this enzyme, but it is uncertain whether the total enzyme has yet been measured in these preparations. One of the two known reversibly inactive forms of the enzyme was identified in such soluble fractions. This was the apoenzyme, which was apparently supplied with hematin leached from the formed elements in homogenates, and in soluble preparations was activated by addition of hematin, without added reduction (1, 3). In whole homogenates, reduction by critical amounts of hydrogen peroxide increased the rate of the tryptophan pyrrolase reaction (4-8) by activating a second reversibly inactive form of the enzyme. Peroxide and also ascorbic acid reduce the inactive ferriporphyrin form of the enzyme that is present in partially purified preparations to the active ferroporphyrin form (9). The combination of added hematin plus reduction was essential to activate a highly purified apotryptophan pyrrolase from liver (10), but the combination of these two different activators has not been tested in soluble extracts that would be suitable for measuring the total enzyme from liver.

The present experiments have identified the active and both inactive forms of tryptophan pyrrolase in soluble liver preparations and have defined the conditions for their assay. The rate of the tryptophan pyrrolase reaction in preparations from normal liver was more than doubled by either reduction with peroxide or by addition of hematin, and increased more than the sum of these separate effects with the two activators in combination. Ascorbic acid was a less effective reductant. The critical steady state concentration of hydrogen peroxide for optimal reduction was a calculated concentration of 10⁻⁵ M, maintained by generating it at a particular rate relative to the catalase content of the reaction mixture. Thus, endogenous or added peroxide, hematin, and catalase were identified as the important variables determining whether all or some of the three known enzyme forms would be assayed in soluble preparations. It was possible to measure the proportions of these three forms, and to show that they changed during substrate induction of the enzyme.

EXPERIMENTAL PROCEDURE

Animals and Enzyme Preparations—Male Slonaker (NEDH) rats weighing 230 to 250 g, and male mice of the CD-1 and C57BL strains weighing 30 to 40 g were used. Rats used for induction of the tryptophan pyrrolase were given 50 mg of L-tryptophan intraperitoneally per 100 g of body weight and killed 2 hours later, when activation by hematin was reported to be minimal (3), or 1.5 mg of hydrocortisone (free alcohol) intraperitoneally per 100 g of body weight and killed 5 hours later. Rat livers were homogenized in 4 volumes of cold 0.14 M KCl-0.005 M NaOH mixture with a motor-driven Teflon pestle, and the homogenate was centrifuged at 105,000 × g in a Spinco model L preparative ultracentrifuge for 60 minutes in the cold to remove the hematin-bearing microsomal elements (1). The resulting supernatant solutions were used within 5 hours after preparation for the tryptophan pyrrolase assay or for preparation of the pH 5.4 precipitated fraction. For the latter, an aliquot at 0° was adjusted to pH 5.4 with about 0.02 volume of 0.7 M acetic acid. After rapid centrifugation, the supernatant solution was drawn off, and the precipitate was emulsified with 0.05 of the original volume of 0.2 M NaHPO₄, diluted to half the original volume, and then adjusted to pH 7 with 1 N NaOH as previously described (5). Mouse livers were handled similarly, but were homogenized in 9 volumes of the KCl-NaOH solution and centrifuged at 13,000 × g.

Enzyme Assay—The tryptophan pyrrolase reaction was followed by two methods, one based on the removal of aliquots at 20-minute intervals during aerobic incubation at 37° followed by determination of the kynurenine in the neutralized filtrates (1, 2), and the other with continuous recording in a Beckman model DK spectrophotometer (1) at 390 μm of the optical density of kynurenine formed during the reaction at 25° or 37°. The reaction rates in the 25° assay were 40% of those in the 37° assay. The rates in both assays were similarly affected by the various conditions tested.

The 4-ml assay mixtures consisted of additions in this order: 1 ml of 0.2 M sodium phosphate (pH 7.0), water, 4 μmoles of hematin, liver enzyme preparation, 9 μmoles of L-tryptophan, glucose oxidase, catalase when used, and 200 μmoles of glucose. Tryptophan was omitted from the blank reactions. Freshly
dissolved and neutralized ascorbic acid (usually 40 μmoles) was added in place of glucose oxidase and glucose. Hematin (1.3 mg) was freshly dissolved in 10 ml of 0.1 N NaOH, diluted to 100 ml, and 0.2 ml of this 20 μM solution was added to the assay. The volumes of liver preparations used were 1 ml in the interval assays at 37° and 0.5 ml in the direct assays at 25° or 37°. The same conditions were used for assays of the pH 5.4 precipitated enzyme, except that the amount of ascorbic acid was halved, and 1.0 (absorbance) unit of catalase-free kynurenine formylase (arylformylamine amidohydrolase, EC 3.5.1.9) (11) was added.

The tryptophan pyrrolase activity, expressed as micromoles of kynurenine formed in an hour per ml of enzyme or g of liver, was determined from the linear portion of the rate curves. Projection of this linear portion of the curve onto the time axis provided the measure of the lag phase of the reaction (the period from zero time until the reaction reached its full rate).

Glucose oxidase (Dee-O, from Takamine Laboratories, Clifton, New Jersey) was obtained catalase-free by precipitation with basic lead acetate and ammonium sulfate precipitation (12). One unit catalyzed the uptake of 1 μl of O2 in 10 minutes at 37° in air and in the presence of 0.3 M glucose, 0.07 M phosphate (pH 5.6), 10 μg of catalase, and 0.005 M sodium verenate in a total volume of 3 ml. Its activity measured in the presence of the liver enzyme preparation under the conditions of the tryptophan pyrrolase assay at 37° closely approximated its activity in the standard assay (formation by 1 unit of 1.5 × 10−10 M H2O2 per liter × sec).

The activity of catalase in the liver fractions and in the commercial bovine preparation used (Worthington, lyophilized) was measured spectrophotometrically at 25° by the modified method of Beers and Sizer (13). The velocity constant k was calculated with the equation

\[ k = \frac{2.3}{t} \log \frac{X}{N}, \]

where \( X \) was the initial peroxide concentration expressed as optical density at 240 μM and \( X \) was the concentration at time \( t \) in seconds. The catalase activities given are the total amounts present in the assay mixtures.

**RESULTS**

**Activation by Combined Reduction and Hematin Addition**—The major effect of the additions tested was to increase the final linear rate of the tryptophan pyrrolase reaction. This rate was stable for more than an hour, and was reached after a variable lag phase lasting as long as 25 minutes at 25°. Addition of hematin, or of optimal amounts of glucose oxidase added as a source of peroxide, more than doubled the final rate in extracts of normal and hydrocortisone-induced rats (Table I). The effects were less in tryptophan-induced preparations. The activations by hematin and the reducing agents persisted when the two kinds of activators were tested in combination. Their effects were in fact synergistic. In preparations from the three kinds of differently treated rats, the extra activity produced by the combination of hematin and the reducing agent was about 1.4 times the sum of the extra activities they produced separately (Table I, Column 5). Ascorbic acid as reducing agent increased the final rate only one third as much as did glucose oxidase. The absolute activities with hematin plus a reducing agent were constantly 1.4 times higher with glucose oxidase than with ascorbic acid in the three different kinds of preparations.

**Table I**

<table>
<thead>
<tr>
<th>Additions</th>
<th>(1) None</th>
<th>(2) Hematin</th>
<th>(3) Reduction</th>
<th>(4) Hematin + reduction</th>
<th>(5) Synergy (4)/(2) + (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose oxidase</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The final activities of several separate preparations without additions is given in micromoles per hour X g of liver (37°) and with additions as the per cent increase over the basal activity observed. The two or three preparations tested with glucose oxidase (1 unit per catalase k) were among the four or five also tested with ascorbic acid (20 μmoles).

The lag phase of the reaction was similar to that noted in the same kind of preparation by Feigelson and Greengard (1), although it was not eliminated by hematin addition as described by them, and not eliminated by reduction plus hematin. Even longer lag phases occurred in our earlier partially purified preparations, but these were specifically eliminated by reduction (9, 10). Except for hematin, the addition of which to the present preparations did not substantially shorten the lag phase, all other conditions which increased the final reaction rate also proportionately shortened the lag phase. Thus, the lag phase was shorter at 37° than at 25° and shorter with glucose oxidase than with ascorbic acid, but it was not less than 5 minutes under any conditions.

The reaction rates in preparations from both kinds of induced rats were faster than those from noninduced rats, confirming also in these types of assays the familiar increase in activity that occurs with induction. But the preparations did not behave qualitatively the same in the different assays. Those from the tryptophan-induced rats showed the shortest lag phase and the least relative increase in activity with the additions of the activators (Table I). This behavior indicated that a larger proportion of the tryptophan-induced enzyme existed in the active form. The percentages of each of the three identifiable forms of the enzyme, calculated from the observed activities in Table I in each type of assay, are given in Table II. Thus, the activity without addition was taken as the measure of the active ferroporphyrin form (EH1+), the extra activity produced by the reducing agent measured the ferrisporphyrin form (EH1+), and the extra activity produced by adding hematin to the reduced enzyme preparation measured the apo enzyme (E):

\[ E \rightarrow \text{hematin} (H) \rightarrow EH1^+ \rightarrow \text{ reduction} \rightarrow EH1^+ \text{ (activo)} \] (1)

The proportion of the different forms in the tryptophan-induced preparation was clearly different from that in the other two kinds of preparations. There was undoubtedly some endogenous hematin and reduction, which would bias all the estimates in favor of the conjugated and reduced forms. The
more complete reduction by glucose oxidase than by ascorbic acid also indicated relatively more of the ferriporphyrin form in all of the preparations. Yet in assays with either reducing agent, the apoenzyme was less than a third of the total in the tryptophan-induced preparations and more than half the total in the noninduced and hydrocortisone-induced preparations. The large (total) conjugated fraction in the tryptophan-induced preparations confirms the same finding made by Greengard and Feigelson in the absence of reduction (3, 14). But in addition, the conjugated enzyme in the tryptophan-induced preparations was also predominantly in the reduced form, while the smaller conjugated fractions in the other preparations consisted of about equal amounts of the ferro- and ferriporphyrin forms. Relatively more of the enzyme in the tryptophan-induced preparations was therefore in a form that would be measured in an incompletely activated assay system.

**Optimal Steady State Concentration of Peroxide for Activation**—The routine activation of the tryptophan pyrrolase by generation of peroxide with small, optimal amounts of glucose oxidase, as in the foregoing experiments, was difficult in the past because the margin is narrow between ineffective and inhibitory amounts of peroxide for a given preparation, and because the optimal amount varied in different preparations (6-8). The optimal amount was probably related to the catalase content of the reaction mixture, because the tryptophan pyrrolase reaction was inhibited either by eliminating (4) or adding extra (9) catalase. The much larger glucose oxidase requirement for maximal tryptophan pyrrolase activity in liver preparations from rats (7, 8) than from mice (6) was repeated (Fig. 1), and found to be associated with a higher catalase content of the preparations that required more glucose oxidase. The low catalase activity of C57BL mouse liver has been reported before (15).

The role of catalase in determining the peroxide requirement was shown by precipitating the tryptophan pyrrolase of rat liver at pH 5.4, which separated it from catalase. The requirement for glucose oxidase then dropped so that any amount was actually inhibitory. With the stepwise addition of bovine catalase to the assay system, the inhibition was overcome and the optimal amount of glucose oxidase required was raised. When the catalase activity of the original supernatant fraction was restored, the original glucose oxidase requirement was substantially regained (Fig. 2). It was therefore apparent that the tryptophan pyrrolase reaction rate was to a large extent determined by the steady state concentration of peroxide, and that this was maintained by an optimal relation between the rates of peroxide formation by glucose oxidase and removal by endogenous or added catalase.

The optimal ratio between glucose oxidase and catalase was determined in a series of experiments like that shown in Fig. 1. Rat and mouse liver preparations varying 10-fold in their catalase activities (0.58 to 5.72 k) and in their optimal amounts of glucose oxidase required (0.84 to 0.7 units) all showed tryptophan pyrrolase reaction rates that rose and then fell as more glucose oxidase was added. Despite the wide differences in the absolute amounts of glucose oxidase, catalase, and tryptophan pyrrolase

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**TABLE II**

**Calculated proportions of three forms of tryptophan pyrrolase in noninduced and induced rat liver preparations**

The fraction of the total activity (with hematin plus reducing agent, Table I, Column 4) referable to the apoenzyme (E) was calculated from the extra activity produced by hematin addition in the presence of reducing agent (Table I, Columns 4 - 3); the ferroporphyrin enzyme (EH⁺) from the extra activity upon addition of a reducing agent (Table I, Column 3); and the ferroporphyrin enzyme from the activity without addition (Table I, Column 1).

<table>
<thead>
<tr>
<th>Induction of preparations</th>
<th>Apoenzyme (E)</th>
<th>Ferroporphyrin enzyme (EH⁺)</th>
<th>Ferroporphyrin enzyme (EH⁺)</th>
<th>Total conjugated enzyme (EH⁺ + EH⁻)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not induced</td>
<td>56 (59)*</td>
<td>28 (15)</td>
<td>16 (26)</td>
<td>44 (41)</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>50 (60)</td>
<td>30 (14)</td>
<td>20 (37)</td>
<td>50 (41)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>28 (29)</td>
<td>17 (6)</td>
<td>55 (65)</td>
<td>72 (71)</td>
</tr>
</tbody>
</table>

* Figures in parentheses were calculated from the activities with ascorbic acid, and the others with glucose oxidase as reducing agent. The absolute total activities with glucose oxidase were 1.4 times those with ascorbic acid.
activities in the preparations, the maximal tryptophan pyrrolase activity for each preparation occurred near the ratio of 1.0 unit of glucose oxidase per catalase \( k \) (Fig. 3). The ratio was independent of the absolute amounts, because it was also unchanged when one-fourth the usual volumes were assayed.

The absolute amounts of catalase and glucose oxidase did affect the critical character of the optimal ratio. In preparations with the highest absolute amounts of catalase, the rates were maximal over a wider range of glucose oxidase-catalase ratios, and were inhibited less at the higher ratios (Figs. 1 to 3). Since the steady state concentration of peroxide is determined solely by the ratio of the rates of its formation and removal, and not by the absolute amounts of the enzymes concerned, it is probable that the larger amounts of enzyme maintained a more stable concentration of peroxide. It is possible that some catalase or glucose oxidase was inactivated during the reactions. The resulting change in peroxide concentration would be least when the enzyme lost was a small fraction of that present.

The steady state concentrations of hydrogen peroxide in the assay mixtures at maximal tryptophan pyrrolase reaction rates were calculated by equating the instantaneous rate of the zero order production of peroxide by added glucose oxidase (G.O.) with the rate of its first order removal by the catalase (cat) present (16):

\[
\frac{d[H_2O_2]}{dt} = \text{units}_{G.O.} \times 1.5 \times 10^{-10} \text{ M/sec} = [H_2O_2] \times k_{cat} / \text{sec} \\
[H_2O_2] = \text{units}_{G.O.} / k_{cat} \times 1.5 \times 10^{-10} \text{ M}
\]

At the ratio of 1.0 unit of glucose oxidase (37°) per catalase \( k \) (25°) that was optimal for all the preparations, the estimated steady state concentration of hydrogen peroxide was 1.5 \( \times \) 10^{-10} M. At 2.5 units per \( k \), the highest ratio that still gave maximal activities in those rat liver preparations with the largest absolute amounts of catalase, the estimated steady state peroxide concentration was 3.8 \( \times \) 10^{-10} M. Since catalase activity was measured at 25° and glucose oxidase at 37°, the peroxide concentrations resulting from both enzymes working at the same temperature would be about half those given (0.7 to 1.9 \( \times \) 10^{-9} M).

**Reductive Activation of Tryptophan Pyrrolase by Ascorbic Acid**—The activation of the partially purified tryptophan pyrrolase of liver and Pseudomonas bacteria by ascorbic acid was qualitatively similar to the activations by peroxide (9). Ascorbic acid caused immediate reduction of the enzyme in the presence of tryptophan to the ferroporphyrin form, identified by its spectrum and by its CO derivative (9). It was not determined at that time whether ascorbic acid reduced the tryptophan pyrrolase directly, or whether it first formed hydrogen peroxide that reduced the enzyme. This was tested in a rat liver preparation that was rich in catalase, and that was significantly activated by 5 or more units of glucose oxidase (Fig. 4). The same preparation, after precipitation at pH 5.4 to remove the catalase, was completely inhibited by 2 or more units of glucose oxidase, but 5 or more 

![Fig. 3. The relative tryptophan pyrrolase (TP) activities at 37° in 1 ml of various rat and mouse liver preparations. Increasing amounts of added glucose oxidase are expressed as the ratio of added units to catalase \( k \) in the assay mixture. Points on the ordinate are with no added glucose oxidase. The preparations and their absolute catalase activities were: Curve 1, average of six rat supernatant fractions (5.72 \( k \)); Curve 2, rat pH 5.4 precipitate plus catalase (5.9 \( k \)); Curve 3, CD-1 mouse supernatant fraction (5.40 \( k \)); Curve 4, same as Curve 2 with less catalase (0.97 \( k \)); Curve 5, C57BL mouse supernatant fraction (0.84 \( k \)).](http://www.jbc.org/content/241/10/2219/F3)

![Fig. 4. Comparison of the reductive activation of tryptophan pyrrolase at 37° by glucose oxidase (---) and ascorbic acid (-----) in 1 ml of the supernatant fraction of rat liver (C, catalase \( k = 5.4 \)), and the pH 5.4 precipitate (\( \triangle \), catalase \( k = 0.04 \)).](http://www.jbc.org/content/241/10/2219/F4)

**DISCUSSION**

The separate activations of tryptophan pyrrolase obtained with hematin and reduction, and with each in the presence of the other, demonstrate that they act in different ways in the crude extracts used here. In addition, the two different reversibly inactive forms of tryptophan pyrrolase on which they act must be present in these preparations. Therefore, both kinds...
of activators must be added for quantitative assays of tryptophan pyrrolase in such preparations.

The two different kinds of activation are not entirely independent, but are synergistic. The apoenzyme when combined with hematin forms only the inactive ferriporphyrin enzyme, so the apoenzyme requires hematin plus reduction to be activated. This is the basis of the synergy. The ferriporphyrin enzyme requires only reduction. The demonstrated inactivity of the ferriporphyrin enzyme (9) has been questioned (17), but the combination of purified apoenzyme plus hematin was also demonstrated to be inactive until reduced (10). Activation of the apoenzyme in soluble liver extracts with added hematin but without added reduction has been reported (1, 3, 18). This can be explained in part by the endogenous reduction of most of the newly conjugated ferriporphyrin enzyme that occurs in crude preparations. Some activation occurred in the present experiments with hematin addition without added reduction (Table I) by virtue of endogenous reduction. It amounted to two-thirds of the full hematin effect observed in the presence of reduction. In purified apoenzyme preparations that were activated by hematin addition alone (19), reduction must also have occurred in some way. The effective reduction by only 10^{-10} M peroxide noted here would make it difficult to exclude such reduction without special precautions. In our purified preparations of the same kind, reduction was found to be essential, but some slow endogenous reduction could be demonstrated (10). The increased activities previously observed upon addition of hematin alone were therefore composite activations by hematin plus some degree of endogenous reduction.

The tryptophan-induced enzyme was activated less by added hematin than the noninduced or hydrocortisone-induced enzymes (3, 14). A higher hematin content, or a greater extent of reduction, would have this effect. The present studies showed that the tryptophan-induced enzyme was both more highly conjugated with hematin and more fully reduced than the noninduced and hydrocortisone-induced enzymes. This substantiates the conclusion of Greengard and Feigelson (3, 14) that the substrate-induced enzyme was more fully conjugated, and confirms the basis for their hypothesis that a decrease in the apoenzyme concentration triggers the substrate-induced accumulation of tryptophan pyrrolase.

The persistence of a variable lag phase in the tryptophan pyrrolase reaction in these crude preparations emphasizes the importance of making serial measurements to determine the final reaction rate (1). The duration of this lag in fresh preparations is minimal in whole homogenates of rat liver (20), as long as a half-hour in the soluble fractions, and minimal (19) or indefinitely prolonged (9, 10) in purified enzymes, depending on the rapidity of endogenous reduction. It can be specifically eliminated by addition of a reducing agent to partially (9) and extensively (10), purified enzyme preparations. But the reducing agents only shortened and did not eliminate the lag in the fresh soluble preparations studied here. This persistence of the lag phase suggests that there is a still unrecognized form of the enzyme in the crude preparations which is not immediately activated by hematin plus reduction as are the known purified forms (10), but which is converted to the known forms rapidly in homogenates and slowly in particle-free supernatant fractions.

The main effect of reducing agents here was to increase the final reaction rate. This suggests that the role of reducing agents is not only to accelerate the initial conversion of inactive ferri-hematin addition alone (19), reduction must also be explained in part by the endogenous reduction of most of the newly conjugated ferriporphyrin enzyme that occurs in crude preparations. Some activation occurred in the present experiments with hematin addition without added reduction (Table I) by virtue of endogenous reduction. It amounted to two-thirds of the full hematin effect observed in the presence of reduction. In purified apoenzyme preparations that were activated by hematin addition alone (19), reduction must also have occurred in some way. The effective reduction by only 10^{-10} M peroxide noted here would make it difficult to exclude such reduction without special precautions. In our purified preparations of the same kind, reduction was found to be essential, but some slow endogenous reduction could be demonstrated (10). The increased activities previously observed upon addition of hematin alone were therefore composite activations by hematin plus some degree of endogenous reduction.

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rate of peroxide generation, the concentration of tissue catalase can very significantly affect the measurable tryptophan pyrrolase activity (Figs. 1 and 2). In nonhepatic tissues, and in livers of tumor-bearing animals which frequently have low catalase activities, tryptophan pyrrolase could be present but not measured unless the ratio of peroxide generation to catalase was controlled.

Ascorbic acid was less effective than glucose oxidase as a reductant, but simpler to use. Its reductive action was direct and not through peroxide formation, and high concentrations were not inhibitory. The constancy of its observed effect in the fresh preparations from three kinds of differently treated rats, in which it regularly gave 72% as much activity in combination with hematin as did glucose oxidase, suggests that in these limited situations the results obtained with it would be proportional to the total enzyme content of the tissue as determined with glucose oxidase. Under other conditions, such as its use in homogenates or with added heavy metals (22, 23), its known oxidation to form peroxide (29) could alter its effect and possibly cause inhibition.

**SUMMARY**

Reductive activators and hematin each more than doubled the final rate of the tryptophan pyrrolase reaction in particle-free soluble fractions of normal rat livers. In combination, these activators increased the reaction rate more than the sum of their separate effects. The tryptophan pyrrolase therefore occurs in at least three forms, and the proportions of these were measured: the apoenzyme uncombined with its hematin prosthetic group, the inactive ferriporphyrin enzyme, and the active ferroporphyrin enzyme. The proportion of the latter increased during tryptophan induction. A persistent lag period suggested the presence of a fourth, slowly activated form.

The most effective reductive activator was hydrogen peroxide, generated by glucose oxidase at a fixed rate relative to the catalase activity present. The optimal effect was at a calculated steady state concentration of about 10⁻⁴ M peroxide. Ascorbic acid was a less effective activator, but acted directly and independently of the catalase present. The results illustrate the feasibility of the tryptophan pyrrolase assay in soluble liver fractions, but also the need to control the hematin, peroxide, and catalase concentrations in order to avoid potentially large errors in the estimation of the enzyme concentration from its activity in such preparations.

**Acknowledgment—Dr. Hans M. Eppenberger kindly checked for us the reproducibility of the relative tryptophan pyrrolase activities at 37° with hematin plus optimal glucose oxidase or ascorbic acid in several preparations from hydrocortisone-induced rats. In his experiments, the maximal activities with glucose oxidase also occurred with 1 unit of glucose oxidase per catalase k, and the absolute activities were also 1.4 to 1.5 times those with ascorbic acid.**

**REFERENCES**

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